2:15

407-2 Targeted Expression of Cre Recombinase Provokes Cardiac-restricted, Site-specific Gene Rearrangement in Ventricular Muscle in vivo

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Mouse models for human disease have been enriched by homologous recombination to create germline loss-of-function mutations. However, principal gains from this strategy often pertain to development: "knock outs" have yielded fewer insights into adult cardiac pathophysiology, due to early lethality, and can be confounded by systemic dysfunction in the absence of a vital protein. Site-specific recombination can circumvent these pitfalls, by enabling precise temporal control, or restriction of genetic recombination to a given organ or subset of cells. One such system, using the Cre recombinase from bacteriophage P1, mediates site-specific deletion of DNA between paired loxP sites. Here, we have exploited transgenic mouse technology plus adenoviral gene transfer to achieve Cre-mediated recombination in cardiac muscle cells in vivo: (1) In vitro, a Cre gene driven by cardiac-specific αMHC regulatory sequences elicited recombination selectively at loxP sites in purified cardiac myocytes, but not in cardiac fibroblasts. (2) In vivo, this αMHC-Cre transgene elicited recombination in cardiac muscle, but not other organs, as ascertained by PCR analysis, and by histochemical and immunochemical localization of the Cre-dependent LacZ reporter protein. (3) Adenoviral delivery of Cre in vivo provoked recombination in post-mitotic, adult ventricular muscle cells. (4) There was no evidence for spontaneous, homologous recombination between loxP sites in the absence of Cre, under these conditions. These studies demonstrate the potential utility of Cre-mediated recombination for adult myocardium, where numerous genomic knock-out mutations are embryonic-lethal, and establish the potential for gene recombination in post-mitotic cells more generally. Moreover, direct delivery of the Cre gene provides an unambiguous means to trigger recombination at predetermined times.

2:30

407-3 Enhancement of Transgene Expression in Lipid-loaded Endothelial Cells

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Adenoviral-mediated gene transfer to the endothelium is a potential therapeutic strategy for vascular disease. Previously, we demonstrated that transgene expression in endothelium was greater in atherosclerotic than normal vessels. We hypothesized that this might be due to differences in lipid composition of the endothelial cells. The purpose of this study was to determine why expression of a reporter gene is augmented in lipid-loaded endothelial cells by determining if uptake of the viral vector or expression of the transgene is altered. Exposure of bovine aortic endothelial cells (BAEC) to acetylated LDL (AcLDL) for 24 hours increased cholesterol content from 21 $\pm$ 1 mg/dl (mean $\pm$ SEM) to 46 $\pm$ 9 mg/dl ($p < 0.05$). Normal and lipid-loaded BAEC were transfected with an adenoviral vector (MOI = 1) containing the gene for β-galactosidase and regulated by the CMV major immediate early promoter (Ad2/CMVβgal). β-gal activity was assessed using a chemiluminescent assay (Galactolyte). There was a 400-fold increase in β-gal activity in lipid-loaded cells, compared to control cells ($p < 0.05$). Quantification of transfected DNA by Southern blot analysis demonstrated no significant difference between control and lipid-loaded BAEC. By comparison, there was an increase in β-galactosidase mRNA in lipid-loaded cells, compared to normal cells. There also was increased expression of the DNA binding activity of transcription factors NFκB and CREB, that are known to increase expression of the CMV promoter, in lipid-loaded cells. We conclude that transgene expression, rather than uptake of the viral vector, is increased in lipid-loaded cells. We speculate that the major immediate early promoter of CMV may be useful to target transgene expression to atherosclerotic vessels.

2:45

407-4 Gax in Cardiogenesis: A Homeobox Protein Developmentally Expressed in all Three Muscle Lineages

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The homeobox gene *Gax* was initially detected in adult cardiovascular tissues and exhibits a growth arrest-specific pattern of expression in cultured vascular myocytes. While the purpose of several other transcription factors, including Nkx2.5/Csx/Nkx2.6, Gata-IV, MEF2 and the HAND genes have been described in the developing mammalian heart, the role Gax may play in the morphogenetic events of cardiac muscle development remains to be elucidated.

Immunohistochemical and *in situ* analysis in mouse embryos and protein expression in chick embryos during cardiac embryogenesis revealed early protein expression within the lateral plate mesoderm. Gax was present during early heart tube formation in both mice and chick embryos and, during segmentation, was present throughout the common ventricle, bulbus cordis and atria. Peak protein expression appeared in cardiac muscle at the septation and looping stages of development. Immediately thereafter, Gax protein was down-regulated, offset from *gax* mRNA by one day, and became detectable again within the compact layer of the ventricles during the latter stages of embryogenesis. Gax was not detected at any point during cardiogenesis in the endocardium, the epicardium or any region of the endocardial cushions or interatrial septa. Western blot analysis at days 12.5, 13.5 and 15.5 days p.c. revealed a single band, consistent with Gax's known coding sequences. Overexpression of Gax in chick embryos resulted in truncation of the common ventricle and rounding of the bulbus cordis and LV outflow tract consistent with alterations in cardiomyocyte migration or proliferation. MyoD transgenic mice using an MCK promoter induced precocious and sustained Gax mRNA and protein expression. Moreover, the biphasic pattern of Gax expression in the heart was abrogated, consistent with the transactivation through a consensus MEF2 regulatory site. These data suggest an important developmental role for Gax in cardiac muscle development and perhaps all myogenic lineages via reciprocal transactivation between Gax, bHLH proteins and MEF2 factors.

3:00

407-5 β_3 Integrins are Upregulated Following Vascular Injury and Mediate Proliferation of Cultured Smooth Muscle Cells

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In The Evaluation of c7E3 to Prevent Ischemic Complications (EPIC) trial, treatment with an antibody that binds β_3 integrins (abciximab;c7E3 Fab) at the time of coronary angioplasty reduced the need for repeat revascularization. Two potential mechanisms have been proposed to explain this effect: 1) inhibition of platelet aggregation; or 2) interruption of ligand binding to β_3 integrins on the smooth muscle cell (SMC) surface. We examined the latter hypothesis by determining if: 1) expression of β_3 integrins within the vessel wall is upregulated following balloon angioplasty in the baboon, 2) expression of thrombospondin (TSP), a potential β_3 ligand, is upregulated following vascular injury, 3) 7E3 binds β_3 integrins on cultured SMC and 4) β_3 integrin activation plays a role in TSP-induced signal transduction and/or proliferation of cultured SMC. Results demonstrated that staining for β_3 integrins was undetectable in uninjured arteries but present perilumenally and throughout the neointima one week following injury. Staining for TSP markedly increased and was present throughout the neointima following injury. 7E3 bound to cultured human aortic SMC with high affinity and this binding was competitively inhibited by LM609, a monoclonal antibody that binds $\alpha_v\beta_3$ with high specificity. Cotreatment of cultured SMC with 7E3 reduced TSP-induced increases in mitogen associated protein kinase (MAP-kinase) activity and proliferation. In summary, these studies demonstrate that expression of β_3 integrins increases following vascular injury and support the hypothesis that β_3 integrins play a role in SMC growth responses.